

FORM PTO-1390 (REV. 10-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 20332P
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/831580
INTERNATIONAL APPLICATION NO. PCT/US99/26303	INTERNATIONAL FILING DATE 08 November 1999	PRIORITY DATE CLAIMED 12 November 1998	
TITLE OF INVENTION G PROTEIN-COUPLED RECEPTOR RESEMBLING THE LEUKOTRIENE B4 RECEPTOR			
APPLICANT(S) FOR DO/EO/US QINGYUN LIU, RUIPING WANG, WENDY J. BAILEY AND MICHAEL DAVIDOFF			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.			
2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.			
3. <input type="checkbox"/> This is an express request to begin national examination procedures [35 U.S.C. 371(f)] at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(l).			
<input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made and the US was elected by the expiration of the 19th month from the earliest claimed priority date (PCT Article 31).			
<input checked="" type="checkbox"/> A copy of the International Application as filed [35 U.S.C. 371(c)(2)].			
a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).			
b. <input type="checkbox"/> has been communicated by the International Bureau.			
c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).			
6. <input type="checkbox"/> An English language translation of the International Application as filed [35 U.S.C. 371(c)(2)].			
<input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)].			
a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).			
b. <input type="checkbox"/> have been communicated by the International Bureau.			
c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.			
d. <input type="checkbox"/> have not been made and will not be made.			
8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)].			
9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)].			
10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)].			
Items 11 to 16 below concern other document(s) or information included:			
11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.			
12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.			
13. <input type="checkbox"/> A FIRST preliminary amendment.			
<input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.			
14. <input type="checkbox"/> A substitute specification.			
15. <input type="checkbox"/> A change of power of attorney and/or address letter.			
16. <input type="checkbox"/> Other items or information:			

EXPRESS MAIL CERTIFICATE

DATE OF DEPOSIT May 11, 2001

EXPRESS MAIL NO. EP 392097111US

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS
BEING DEPOSITED WITH THE UNITED STATES POSTAL
SERVICE AS EXPRESS MAIL "POST OFFICE TO
ADDRESSEE" BEFORE 5 P.M. ON THE ABOVE DATE IN
AN ENVELOPE ADDRESSED TO ASSISTANT COMMISSIONER
FOR PATENTS, WASHINGTON, D.C. 20231.

MAILED BY A. B. Crowley

DATE 5/11/01

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)		INTERNATIONAL APPLICATION NO		ATTORNEY'S DOCKET NUMBER	
09/831580		PCT/US99/26303		20332P	

17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE [37 CFR 1.492(a)(1)-(5)]: Neither international preliminary examination fee (37 CFR 1.482) nor international search fee [37 CFR 1.445(a)(2)] paid to USPTO and International Search Report not prepared by the EPO or JPO..... \$1,000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... \$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee [37 CFR 1.445(a)(2)] paid to USPTO..... \$710.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... \$690.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... \$100.00 <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>				CALCULATIONS	PTO USE ONLY

Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date [37 CFR 1.492(e)].				\$0.00	
---	--	--	--	--------	--

Claims	Number Filed	Number Extra	Rate		
Total Claims	13 - 20 =	0	X \$18.00	\$0.00	
Independent Claims	6 - 3 =	3	X \$80.00	\$240.00	
Multiple dependent claim(s) (if applicable)		0	+ \$270.00	\$0.00	
TOTAL OF ABOVE CALCULATIONS =				\$340.00	

<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.					
SUBTOTAL =				\$340.00	

Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date [37 CFR 1.492(f)].				+	\$0.00
TOTAL NATIONAL FEE =				\$340.00	

Fee for recording the enclosed assignment [37 CFR 1.21(h)]. The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property.				+	
TOTAL FEES ENCLOSED =				\$340.00	
				Amount to be refunded	
				charged	

a. ☐ A check in the amount of \$ _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 13-2755 in the amount of \$340.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to the Deposit Account No. 13-2755. A duplicate copy of this sheet is enclosed.

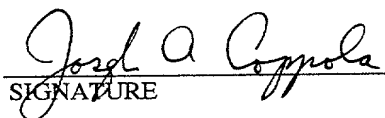
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive [37 CFR 1.137(a) or (b)] must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

MERCK & CO., INC.
 Patent Department, RY60-30
 P.O. Box 2000
 126 East Lincoln Avenue
 Rahway, New Jersey 07065-0970

DATE: MAY 11, 2001

PHONE #: (732) 594-6734


 SIGNATURE
Joseph A. Coppola
 NAME
38,413
 REGISTRATION NUMBER

PTO/PCT Rec'd 11 MAY 2001

TITLE OF THE INVENTION

G PROTEIN-COUPLED RECEPTOR RESEMBLING THE LEUKOTRIENE B4
RECEPTOR

5 CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

10

REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

15

This invention relates to a novel human DNA encoding HG07, a G protein-coupled receptor (GPCR) related to the leukotriene B4 receptor, the protein encoded by the DNA, and methods of identifying selective agonists and antagonists of the protein encoded by the DNA.

20 BACKGROUND OF THE INVENTION

G-protein coupled receptors (GPCRs) are a very large class of membrane receptors that relay information from the exterior to the interior of cells. GPCRs function by interacting with a class of heterotrimeric proteins known as G-proteins. Most GPCRs function by a similar mechanism. Upon the binding of
25 agonist, a GPCR catalyzes the dissociation of guanosine diphosphate (GDP) from the α subunit of G proteins. This allows for the binding of guanosine triphosphate (GTP) to the α subunit, resulting in the disassociation of the α subunit from the β and γ subunits. The freed α subunit then interacts with other cellular components, and in the process passes on the extracellular signal represented by the presence of the
30 agonist. Occasionally, it is the freed β and γ subunits which transduce the agonist signal.

GPCRs possess common structural characteristics. They have seven hydrophobic domains, each about 20-30 amino acids long, linked by sequences of hydrophilic amino acids of varied length. These seven hydrophobic domains
35 intercalate into the plasma membrane, giving rise to a protein with seven

transmembrane domains, an extracellular amino terminus, and an intracellular carboxy terminus (Strader et al., 1994, Ann. Rev. Biochem. 63:101-132; Schertler et al., 1993, Nature 362:770-772; Dohlman et al., 1991, Ann. Rev. Biochem. 60:653-688).

5 GPCRs are expressed in a wide variety of tissue types and respond to a wide range of ligands, *e.g.*, protein hormones, biogenic amines, peptides, lipid derived messengers, *etc.* Given their wide range of expression and ligands, it is not surprising that GPCRs are involved in many pathological states. This has led to great interest in developing modulators of GPCR activity that can be used pharmacologically. For
10 example, Table 1 of Stadel et al., 1997, Trends Pharmacol. Sci. 18:430-437, lists 37 different marketed drugs that act upon GPCRs. Accordingly, there is a great need to understand GPCR function and to develop agents that can be used to modulate GPCR activity.

Leukotriene B4 (5(S),12(R)-dihydroxy-6,14-*cis*, 8,10,-*trans*-
15 eicosatetraenoic acid) (LTB4) is involved in various immune responses, such as defenses against infection, and is involved in the process of inflammation as well. LTB4 induces chemotactic migration (Goetzl & Pickett, 1980, J. Immunol. 125:1789-1791) and chemokinetic activity (Palmer et al., 1980, Prostaglandins 20:411-418) as well as aggregation of polymorphonuclear leukocytes (Ford-Hutchinson et al., 1980,
20 Nature 286:264-265).

High levels of LTB4 are implicated in certain disorders involving inflammation, such as psoriasis (Degiulio et al., 1993, Ann. New York Acad. Sci. 685:614-617). LTB4 has been detected in the spinal fluid of multiple sclerosis patients (Neu et al., 1992, Acta Neurol. Scand. 86:586-587) and inhibition of the
25 interaction of LTB4 with its receptor prevents the development of paralysis and the infiltration into the spinal chord by eosinophils in experimental allergic encephalomyelitis, a murine model of multiple sclerosis (Gladue et al., 1996, J. Exp. Med. 183:1893-1898).

Yokomizo et al., 1997, Nature 387:620-624 isolated a human cDNA
30 encoding a receptor for LTB4 from HL-60 cells that had been stimulated to differentiate by retinoic acid. Based upon its deduced amino acid sequence (GenBank accession numbers D89078 [cDNA]; D89079 [protein]), this cDNA encodes a seven-transmembrane domain G-protein coupled receptor. Transfection of this cDNA into cells not normally expressing the encoded receptor, and not normally responsive to
35 leukotriene B4, resulted in the conversion of the transfected cells into cells that

specifically bound LTB₄ with a K_d similar to that of differentiated HL-60 cells. Upon exposure to LTB₄, the transfected cells also displayed several functional responses characteristic of LTB₄-responsive cells: an increase in intracellular calcium, D-*myo*-inositol 1,4,5-triphosphate (InsP₃) accumulation, inhibition of adenylyl cyclase, and chemotaxis.

SUMMARY OF THE INVENTION

The present invention is directed to a novel human DNA that encodes a G-protein coupled receptor, HG07. The DNA encoding HG07 is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:1. Also provided is an HG07 protein encoded by the novel DNA sequence. The HG07 protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:2. Methods of expressing HG07 in recombinant systems and of identifying agonists and antagonists of HG07 are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the complete cDNA sequence of HG07 (SEQ.ID.NO.:1).

Figure 2 shows the complete amino acid sequence of HG07 (SEQ.ID.NO.:2).

Figure 3A-B shows the location of the HG07 open reading frame. The nucleotide sequence shown is a portion of (SEQ.ID.NO.:1). The amino acid sequence shown is (SEQ.ID.NO.:2).

Figure 4 shows the results of a Northern blot of HG07 mRNA in various human tissues.

Figure 5 shows an alignment of the amino acid sequence of HG07 with the amino acid sequence of a human leukotriene B₄ receptor (SEQ.ID.NO.:3; GenBank accession number D89079, see Yokomizo et al., 1997, Nature 387:620-624).

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

"Substantially free from other proteins" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins.

Thus, an HG07 protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-HG07 proteins. Whether a given HG07 protein preparation is substantially
5 free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting.

"Substantially free from other nucleic acids" means at least
10 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. Thus, an HG07 DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%,
15 of non-HG07 nucleic acids. Whether a given HG07 DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

"Functional equivalent" means a receptor which does not
20 have exactly the same amino acid sequence as naturally occurring HG07, due to alternative splicing, substitutions, deletions, mutations, or additions, but retains substantially the same biological activity as HG07. Such functional equivalents will have significant amino acid sequence
25 identity with naturally occurring HG07. Genes and DNA encoding such functional equivalents can be detected by reduced stringency hybridization with a DNA sequence encoding naturally occurring HG07. For the purposes of this invention, naturally occurring HG07 has the amino acid sequence shown as SEQ.ID.NO.:2 and is encoded by
30 SEQ.ID.NO.:1. A nucleic acid encoding a functional equivalent has at least about 50% identity at the nucleotide level to SEQ.ID.NO.:1.

A polypeptide has "substantially the same biological activity" as HG07 if that polypeptide has a K_d for a ligand that is no more than 5-fold greater than the K_d of HG07 having SEQ.ID.NO.:2 for the same ligand.

A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (e.g., arginine for lysine; glutamic acid for aspartic acid).

By "isolated HG07 protein" or "isolated HG07 DNA" is meant HG07 protein or DNA encoding HG07 that has been isolated from a natural source. Use of the term "isolated" indicates that HG07 protein or DNA has been removed from its normal cellular environment. Thus, an isolated HG07 protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated HG07 protein is the only protein present, but instead means that an isolated HG07 protein is at least 95% free of non-amino acid material (e.g., nucleic acids, lipids, carbohydrates) naturally associated with the HG07 protein. Thus, an HG07 protein that is expressed in bacteria or even in eukaryotic cells which do not naturally (*i.e.*, without human intervention) express it through recombinant means is an "isolated HG07 protein." Similarly, DNA encoding HG07 that is present in bacteria or even in eukaryotic cells which do not naturally (*i.e.*, without human intervention) contain it through recombinant means is an "isolated DNA encoding HG07."

One aspect of this invention is an isolated DNA comprising nucleotides encoding a polypeptide having the amino acid sequence SEQ.ID.NO.:2. This isolated DNA is substantially free from other nucleic acids and can be either single stranded or double stranded, *i.e.*, paired with its complementary sequence. Another aspect of this invention is the identification and cloning of a cDNA which encodes HG07, a G protein-coupled receptor. This cDNA is substantially free from other nucleic acids and can be either single stranded or double stranded. The present invention provides a cDNA molecule substantially free from other nucleic acids having the nucleotide sequence shown in Figure 1 as SEQ.ID.NO.:1. SEQ.ID.NO.:1 contains an open reading frame (positions 158-1,324 of SEQ.ID.NO.:1) encoding a protein of 389 amino acids (see Figure 3A-B). Thus, the present invention also

provides a DNA molecule substantially free from other nucleic acids comprising the nucleotide sequence of positions 158-1,324 of SEQ.ID.NO.:1. The present invention also provides recombinant DNA molecules comprising the nucleotide sequence of positions 158-1,324 of SEQ.ID.NO.:1.

5 Based on its predicted amino acid sequence, the HG07 protein most likely represents a novel G-protein coupled receptor (GPCR) since the HG07 protein contains many of the characteristic features of G-protein coupled receptors (GPCRs), *e.g.*.

- 10 (a) seven transmembrane domains;
 (b) three intracellular loops;
 (c) three extracellular loops; and
 (d) the GPCR triplet signature sequence.

15 Northern blot analysis (Figure 4) showed that HG07 RNA is widely expressed in humans as a transcript of about 2.4 kb, especially in cells of the immune system (peripheral blood lymphocytes (PBLs) and spleen). This argues for a role for HG07 in such immune system functions as inflammation, responses to infection, chemotaxis of lymphocytes, *etc.*.

20 The novel DNA sequences of the present invention encoding HG07, in whole or in part, can be linked with other DNA sequences, *i.e.*, DNA sequences to which HG07 is not naturally linked, to form "recombinant DNA molecules" containing HG07. The novel DNA sequences of the present invention can be inserted into vectors in order to direct recombinant expression of HG07. Such vectors may be comprised of DNA or RNA; for most purposes DNA vectors are preferred. Typical
25 vectors include plasmids, modified viruses, bacteriophage, cosmids, yeast artificial chromosomes, and other forms of episomal or integrated DNA that can encode HG07. One skilled in the art can readily determine an appropriate vector for a particular use.

30 Included in the present invention are DNA sequences that hybridize to SEQ.ID.NO.:1 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing
35 100 µg/ml denatured salmon sperm DNA and 5-20 X 10⁶ cpm of ³²P-labeled probe.

Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

- Other procedures using conditions of high stringency would include
- 5 either a hybridization step carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

- Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of
- 10 these reagents can be found in, *e.g.*, Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

- The degeneracy of the genetic code is such that, for all but two amino
- 15 acids, more than a single codon encodes a particular amino acid. This allows for the construction of synthetic DNA that encodes the HG07 protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ.ID.NO.:1, but still encodes the same HG07 protein as SEQ.ID.NO.:1. Such synthetic DNAs are intended to be within the scope of the present invention. If it is
- 20 desired to express such synthetic DNAs in a particular host cell or organism, the codon usage of such synthetic DNAs can be adjusted to reflect the codon usage of that particular host, thus leading to higher levels of expression of HG07 protein in the host.

- Another aspect of the present invention includes host cells that have
- 25 been engineered to contain and/or express DNA sequences encoding HG07. Such recombinant host cells can be cultured under suitable conditions to produce HG07. An expression vector containing DNA encoding HG07 can be used for expression of HG07 in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as
- 30 yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey, and rodent origin, and insect cells including but not limited to, *Drosophila* and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable for recombinant expression of HG07 and which are commercially available, include but are not limited to, L cells L-M(TK⁻) (ATCC CCL

1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86),
CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651),
CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658),
HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and
5 MRC-5 (ATCC CCL 171).

Human embryonic kidney (HEK 293) cells and Chinese hamster ovary
(CHO) cells are particularly suitable for expression of the HG07 protein because these
cells express a large number of G-proteins. Thus, it is likely that at least one of these
G-proteins will be able to functionally couple the signal generated by interaction of
10 HG07 and its ligands, thus transmitting this signal to downstream effectors,
eventually resulting in a measurable change in some assayable component, e.g.,
cAMP level, expression of a reporter gene, hydrolysis of inositol lipids, or
intracellular Ca^{2+} levels.

Other cells that are particularly suitable for expression of the HG07
15 protein are immortalized melanophore pigment cells from *Xenopus laevis*. Such
melanophore pigment cells can be used for functional assays using recombinant
expression of HG07 in a manner similar to the use of such melanophore pigment cells
for the functional assay of other recombinant GPCRs (Graminski et al., 1993, J. Biol.
Chem. 268:5957-5964; Lerner, 1994, Trends Neurosci. 17:142-146; Potenza &
20 Lerner, 1992, Pigment Cell Res. 5:372-378).

A variety of mammalian expression vectors can be used to express
recombinant HG07 in mammalian and other cells. Commercially available
mammalian expression vectors which are suitable include, but are not limited to,
pCR2.1 (Invitrogen), pMC1neo (Stratagene), pSG5 (Stratagene), pcDNAI and
25 pcDNAIamp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC
37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224),
pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146).
For expression in non-mammalian cells, various suitable expression vectors are
known in the art. The choice of vector will depend upon cell type used, level of
30 expression desired, and the like. Following expression in recombinant cells, HG07
can be purified to a level that is substantially free from other proteins by conventional
techniques, e.g., salt fractionation, ion exchange chromatography, size exclusion
chromatography, hydroxylapatite adsorption chromatography, hydrophobic
interaction chromatography, and preparative gel electrophoresis.

The present invention includes HG07 protein substantially free from other proteins. The amino acid sequence of the full-length HG07 protein is shown in Figure 2 as SEQ.ID.NO.:2. Thus, the present invention includes HG07 protein substantially free from other proteins having the amino acid sequence of

5 SEQ.ID.NO.:2.

As with many receptor proteins, it is possible to modify many of the amino acids of HG07, particularly those which are not found in the ligand binding domain, and still retain substantially the same biological activity as the original receptor. Thus this invention includes modified HG07 polypeptides which have

10 amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as HG07. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, *e.g.*, Molecular Biology of the Gene, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells,

15 1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NO.:2 wherein the polypeptides still retain substantially the same biological activity as HG07. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO.:2 wherein the polypeptides still

20 retain substantially the same biological activity as HG07. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in the ligand-binding domain of HG07.

When deciding which amino acid residues of HG07 may be substituted to produce polypeptides that are functional equivalents of HG07, one skilled in the art would be guided by a comparison of the amino acid sequence of HG07 with the amino acid sequences of related proteins, *e.g.*, the human leukotriene B4 receptor disclosed in Yokomizo *et al.*, 1997, Nature 387:620-624. See also Figure 5 of the

30 present application. Such a comparison would allow one skilled in the art to minimize the number of amino acid substitutions made in regions that are highly conserved between HG07 and the related protein. Accordingly, the present invention includes embodiments where the substitutions are conservative and do not occur in positions where HG07 and the human leukotriene B4 receptor share the same amino

35 acid (see Figure 5).

One skilled in the art would also recognize that polypeptides that are functional equivalents of HG07 and have changes from the HG07 amino acid sequence that are small deletions or insertions of amino acids could also be produced by following the same guidelines, *i.e.*, minimizing the differences in amino acid sequence between HG07 and related proteins. Small deletions or insertions are generally in the range of about 1 to 5 amino acids. The effect of such small deletions or insertions on the biological activity of the modified HG07 polypeptide can easily be assayed by producing the polypeptide synthetically or by making the required changes in DNA encoding HG07 and then expressing the DNA recombinantly and assaying the protein produced by such recombinant expression. Assays that could be used include simple binding assays to determine if the modified HG07 polypeptide is capable of binding the same ligands, with approximately the same affinity, as the naturally occurring HG07 protein. Alternatively, one can use functional assays such as assays for increases in intracellular calcium, D-*myo*-inositol 1,4,5-triphosphate (InsP₃) accumulation, inhibition of adenylyl cyclase, or chemotaxis. Such assays can be based upon similar assays for the leukotriene B₄ receptor disclosed in Yokomizo *et al.*, 1997, *Nature* 387:620-624.

The present invention also includes C-terminal truncated forms of HG07, particularly those which encompass the extracellular portion of the receptor, but lack the intracellular signaling portion of the receptor. Such truncated receptors are useful in various binding assays described herein, for crystallization studies, and for structure-activity-relationship studies.

The present invention also includes chimeric HG07 proteins. Chimeric HG07 proteins consist of a contiguous polypeptide sequence of HG07 fused in frame to a polypeptide sequence of a non-HG07 protein. For example, the N-terminal domain and seven transmembrane spanning domains of HG07 fused at the C-terminus in frame to a G protein would be a chimeric HG07 protein.

The present invention also includes HG07 proteins that are in the form of multimeric structures, *e.g.*, dimers. Such multimers of other G-protein coupled receptors are known (Hebert *et al.*, 1996, *J. Biol. Chem.* 271, 16384-16392; Ng *et al.*, 1996, *Biochem. Biophys. Res. Comm.* 227, 200-204; Romano *et al.*, 1996, *J. Biol. Chem.* 271, 28612-28616).

The present invention also includes isolated forms of HG07 proteins.

The present invention includes methods of identifying compounds that specifically bind to HG07 protein, as well as compounds identified by such methods.

09331560-093301

The specificity of binding of compounds having affinity for HG07 is shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from such cells. Expression of the cloned receptor and screening for compounds that bind to HG07 or that inhibit the binding of a known
5 ligand of HG07 to such cells, or membranes prepared from such cells, provides an effective method for the rapid selection of compounds with high affinity for HG07. Such ligands or compounds can be radiolabeled, but can also be nonisotopic compounds that can be used to displace bound radiolabeled ligands or that can be used as activators in functional assays. Compounds identified by the above method
10 are likely to be agonists or antagonists of HG07 and may be peptides, proteins, or non-proteinaceous organic molecules.

Therefore, the present invention includes assays by which HG07 agonists and antagonists may be identified. Methods for identifying agonists and antagonists of other receptors are well known in the art and
15 can be adapted to identify agonists and antagonists of HG07. Accordingly, the present invention includes a method for determining whether a substance is a potential agonist or antagonist of HG07 that comprises:

- (a) transfecting cells with an expression vector encoding HG07;
- 20 (b) allowing the transfected cells to grow for a time sufficient to allow HG07 to be expressed;
- (c) exposing the cells to a labeled ligand of HG07 in the presence and in the absence of the substance;
- (d) measuring the binding of the labeled ligand to HG07;
- 25 where if the amount of binding of the labeled ligand is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of HG07.

The conditions under which step (c) of the method is practiced are conditions that are typically used in the art for the study of
30 protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

The present invention also includes a method for determining whether a substance is capable of binding to HG07, *i.e.*, whether the

substance is a potential agonist or an antagonist of HG07, where the method comprises:

- (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG07 in the cells;
- 5 (b) exposing the test cells to the substance;
- (c) measuring the amount of binding of the substance to HG07;
- (d) comparing the amount of binding of the substance to HG07 in the test cells with the amount of binding of the substance to control cells that have not been transfected with HG07;

wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to HG07. Determining whether the substance is an agonist or antagonist can then be accomplished by the use of functional assays such as, *e.g.*, the assay involving the use of promiscuous G-proteins described below.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described methods, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) or MRC-5 (ATCC CCL 171).

The assays described above can be carried out with cells that have been transiently or stably transfected with HG07. Transfection is meant to include any method known in the art for introducing HG07 into the test cells. For example, transfection includes calcium phosphate or

calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing HG07, and electroporation.

Where binding of the substance or agonist to HG07 is measured, such binding can be measured by employing a labeled
5 substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, *e.g.*, radioactively, fluorescently, enzymatically.

In particular embodiments of the above-described methods, HG07 has an amino acid sequence of SEQ.ID.NO.:2.

10 The above-described methods can be modified in that, rather than exposing the test cells to the substance, membranes can be prepared from the test cells and those membranes can be exposed to the substance. Such a modification utilizing membranes rather than cells is well known in the art and is described in, *e.g.*, Hess *et al.*, 1992, Biochem. Biophys.
15 Res. Comm. 184:260-268.

Accordingly, the present invention provides a method for determining whether a substance is capable of binding to HG07 comprising:

- 20 (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG07 in the cells;
- (b) preparing membranes containing HG07 from the test cells and exposing the membranes to a ligand of HG07 under conditions such that the ligand binds to the HG07 in the membranes;
- (c) subsequently or concurrently to step (b), exposing the
25 membranes from the test cells to a substance;
- (d) measuring the amount of binding of the ligand to the HG07 in the membranes in the presence and the absence of the substance;
- (e) comparing the amount of binding of the ligand to HG07 in the
30 membranes in the presence and the absence of the substance where a decrease in the amount of binding of the ligand to HG07 in the membranes in the presence of the substance indicates that the substance is capable of binding to HG07;

where HG07 has an amino acid sequence of SEQ.ID.NO.:2.

The present invention provides a method for determining whether a substance is capable of binding to HG07 comprising:

- (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG07 in the cells;
- (b) preparing membranes containing HG07 from the test cells and exposing the membranes from the test cells to the substance;
- 5 (c) measuring the amount of binding of the substance to the HG07 in the membranes from the test cells;
- (d) comparing the amount of binding of the substance to HG07 in the membranes from the test cells with the amount of binding of the substance to membranes from control cells that have not been transfected with HG07;
- 10 where HG07 has an amino acid sequence of SEQ.ID.NO.:2;
- where if the amount of binding of the substance to HG07 in the membranes from the test cells is greater than the amount of binding of the substance to the membranes from the control cells, then the substance is capable of binding to HG07.

15 As a further modification of the above-described methods, RNA encoding HG07 can be prepared, *e.g.*, by *in vitro* transcription using a plasmid containing HG07 under the control of a bacteriophage T7 promoter, and the RNA can be microinjected into *Xenopus* oocytes in order to cause the expression of HG07 in the oocytes. Substances are then

20 tested for binding to the HG07 expressed in the oocytes. Alternatively, rather than detecting binding, the effect of the substances on the electrophysiological properties of the oocytes can be determined.

The present invention includes assays by which HG07 agonists and antagonists may be identified by their ability to stimulate or

25 antagonize a functional response mediated by HG07. One skilled in the art would be familiar with a variety of methods of measuring the functional responses of G-protein coupled receptors (see, *e.g.*, Lerner, 1994, Trends Neurosci. 17:142-146 [changes in pigment distribution in melanophore cells]; Yokomizo et al., 1997, Nature 387:620-624 [changes in cAMP or calcium concentration; chemotaxis]; Howard et al., 1996,

30 Science 273:974-977 [changes in membrane currents in *Xenopus* oocytes]; McKee et al., 1997, Mol. Endocrinol. 11:415-423 [changes in calcium concentration measured using the aequorin assay]; Offermanns & Simon,

1995, J. Biol. Chem. 270:15175, 15180 [changes in inositol phosphate levels]).

Accordingly, the present invention provides a method of identifying agonists and antagonists of HG07 comprising:

- 5 (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG07 in the cells;
- (b) exposing the test cells to a substance that is suspected of being an agonist or an antagonist of HG07;
- (c) measuring the amount of a functional response of the
10 test cells that have been exposed to the substance;
- (d) comparing the amount of the functional response exhibited by the test cells with the amount of the functional response exhibited by control cells;

wherein if the amount of the functional response exhibited by the test
15 cells differs from the amount of the functional response exhibited by the control cells, the substance is an agonist or antagonist of HG07;

where the control cells are cells that have not been transfected with HG07 but have been exposed to the substance or are test cells that have not been
exposed to the substance;

20 where HG07 has the amino acid sequence SEQ.ID.NO.:2.

In particular embodiments, the functional response is selected from the group consisting of: changes in pigment distribution in melanophore cells; changes in cAMP or calcium concentration; chemotaxis; changes in membrane currents in *Xenopus* oocytes; and changes in inositol phosphate levels.

25 HG07 belongs to the class of proteins known as G-protein coupled receptors (GPCRs). GPCRs transmit signals across cell membranes upon the binding of ligand. The ligand-bound GPCR interacts with a heterotrimeric G-protein, causing the $G\alpha$ subunit of the G-protein to disassociate from the $G\beta$ and $G\gamma$ subunits. The $G\alpha$ subunit can then go
30 on to activate a variety of second messenger systems.

Generally, a particular GPCR is only coupled to a particular type of G-protein. Thus, to observe a functional response from the GPCR, it is necessary to ensure that the proper G-protein is present in the system containing the GPCR. It has been found, however, that there are certain

G-proteins that are "promiscuous." These promiscuous G-proteins will couple to, and thus transduce a functional signal from, virtually any GPCR. See Offermanns & Simon, 1995, J. Biol. Chem. 270:15175, 15180 (Offermanns). Offermanns described a system in which cells are
5 transfected with expression vectors that result in the expression of one of a large number of GPCRs as well as the expression of one of the promiscuous G-proteins $G\alpha 15$ or $G\alpha 16$. Upon the addition of an agonist of the GPCR to the transfected cells, the GPCR was activated and was able, via $G\alpha 15$ or $G\alpha 16$, to activate the β isoform of phospholipase C, leading to
10 an increase in inositol phosphate levels in the cells.

Therefore, by making use of these promiscuous G-proteins as in Offermanns, it is possible to set up functional assays for HG07, even in the absence of knowledge of the G-protein with which HG07 is coupled *in vivo*. One possibility is to create a fusion or chimeric protein composed of
15 the extracellular and membrane spanning portion of HG07 fused to a promiscuous G-protein. Such a fusion protein would be expected to transduce a signal following binding of ligand to the HG07 portion of the fusion protein. Accordingly, the present invention provides a method of identifying antagonists of HG07 comprising:

- 20 (a) providing cells that expresses a chimeric HG07 protein fused at its C-terminus to a promiscuous G-protein;
- (b) exposing the cells to an agonist of HG07;
- (c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG07;
- 25 (d) measuring the level of inositol phosphates in the cells; where a decrease in the level of inositol phosphates in the cells in the presence of the substance as compared to the level of inositol phosphates in the cells in the absence of the substance indicates that the substance is an antagonist of HG07.

Another possibility for utilizing promiscuous G-proteins in
30 connection with HG07 includes a method of identifying agonists of HG07 comprising:

- (a) providing cells that expresses both HG07 and a promiscuous G-protein;

(b) exposing the cells to a substance that is a suspected agonist of HG07;

(c) measuring the level of inositol phosphates in the cells; where an increase in the level of inositol phosphates in the
5 cells as compared to the level of inositol phosphates in the cells in the absence of the suspected agonist indicates that the substance is an agonist of HG07.

Levels of inositol phosphates can be measured by monitoring calcium mobilization. Intracellular calcium mobilization is typically
10 assayed in whole cells under a microscope using fluorescent dyes or in cell suspensions via luminescence using the aequorin assay.

In a particular embodiment of the above-described method, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK-)
15 (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) or MRC-5 (ATCC CCL 171).

20 In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of HG07 and the promiscuous G-protein in the cells.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of
25 protein-ligand interactions: e.g., physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of Gα15 or
30 Gα16. Expression vectors containing Gα15 or Gα16 are known in the art. See, e.g., Offermanns; Buhl *et al.*, 1993, FEBS Lett. 323:132-134; Amatruda *et al.*, 1993, J. Biol. Chem. 268:10139-10144.

The above-described assay can be easily modified to form a method to identify antagonists of HG07. Such a method is also part of the present invention and comprises:

- (a) providing cells that expresses both HG07 and a promiscuous G-protein;
- (b) exposing the cells to a substance that is an agonist of HG07;
- (c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG07;
- (d) measuring the level of inositol phosphates in the cells; where a decrease in the level of inositol phosphates in the cells in the presence of the suspected antagonist as compared to the level of inositol phosphates in the cells in the absence of the suspected antagonist indicates that the substance is an antagonist of HG07.

In a particular embodiment of the above-described method, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

The conditions under which steps (b) and (c) of the method are practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of HG07 and the promiscuous G-protein in the cells.

In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of Gα15 or Gα16.

In particular embodiments of the above-described methods, HG07 has an amino acid sequence of SEQ.ID.NO.:2.

Another functional assay that can be used to identify agonists and antagonists of HG07 relies on the ability of leukotriene B4 to induce the chemotaxis or chemokinesis of certain cell types through action at the leukotriene receptor (see, 5 *e.g.*, Yokomizo et al., 1997, *Nature* 387:620-624; Ng et al., 1991, *J. Immunol.* 147:3096-3103). Given the high degree of similarity in amino acid sequence between HG07 and the leukotriene B4 receptor (about 46%, see Figure 5), is is expected that agonists of HG07 will be able to induce the chemotaxis or chemokinesis 10 of cells.

Accordingly, the present invention provides a method of identifying agonists of HG07 comprising:

- (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG07 in the cells;
- 15 (b) exposing the test cells to a substance;
- (c) measuring the amount of chemotaxis or chemokinesis exhibited by the test cells that have been exposed to the substance;
- (d) comparing the amount of chemotaxis or chemokinesis exhibited by the test cells with the amount of chemotaxis or chemokinesis 20 exhibited by control cells;

wherein if the amount of chemotaxis or chemokinesis exhibited by the test cells is greater than the amount of chemotaxis or chemokinesis exhibited by control cells, the substance is an agonist of binding to HG07;

where the control cells are cells that have not been transfected with 25 HG07 but have been exposed to the substance or are test cells that have not been exposed to the substance;

where HG07 has the amino acid sequence SEQ.ID.NO.:2.

In particular embodiments the cells are CHO cells.

While the above-described methods are explicitly directed to testing 30 whether "a" substance is an agonist or antagonist of HG07, it will be clear to one skilled in the art that such methods can be adapted to test collections of substances, *e.g.*, combinatorial libraries, to determine whether any members of such collections are activators or inhibitors of HG07. Accordingly, the use of collections of substances, or individual members of such collections, as the substance in the above- 35 described methods is within the scope of the present invention.

Agonists and antagonists of HG07 that are identified by the above-described methods are expected to have utility in the treatment of diseases that involve the inappropriate expression of HG07. In particular, given the expression pattern of HG07 (see Figure 4), such agonists and antagonists are expected to have utility in the treatment of various immune system disorders or disorders involving inflammation, *e.g.*, multiple sclerosis, arthritis, asthma, lupus. Such agonists and antagonists are also expected to be useful in treating various infectious diseases.

Given the resemblance between HG07 and the leukotriene B₄ (LTB₄) receptor, it is expected that agonists and antagonists of HG07 will have pharmacological activity and be useful in a manner similar to that in which agonists and antagonists of the LTB₄ receptor are useful. Agonists and antagonists of the LTB₄ receptor are useful in the treatment of rheumatoid arthritis, gout, psoriasis, inflammatory bowel disease (U.S. Patent No. 5,684,162), chronic lung diseases, endotoxic shock, septic shock, and adult respiratory distress syndrome (U.S. Patent No. 5,552,441).

While HG07 appears to be most similar to the LTB₄ receptor, and thus is expected to be pharmacologically useful in a manner similar to that of the LTB₄ receptor, it is also expected that HG07 may have affinity for other related leukotrienes, *e.g.*, leukotrienes A₄, C₄, D₄, and E₄. Antagonists of the leukotriene D₄ receptor, such as zafirlukast, montelukast, and pranlukast, have been shown to have utility in the treatment of asthma (Tan & Spector, July/August 1997, Science and Medicine 26-33). Thus, it is expected that antagonists of HG07 may also have utility in the treatment of asthma.

The present invention includes pharmaceutical compositions comprising agonists and antagonists of HG07. The agonists and antagonists are generally combined with pharmaceutically acceptable carriers to form pharmaceutical compositions. Examples of such carriers and methods of formulation of pharmaceutical compositions containing agonists and antagonists and carriers can be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable

composition suitable for effective administration, such compositions will contain a therapeutically effective amount of the agonists and antagonists.

Therapeutic or prophylactic compositions are administered to an individual in amounts sufficient to treat or prevent conditions where
5 HG07 activity is abnormal. The effective amount can vary according to a variety of factors such as the individual's condition, weight, gender, and age. Other factors include the mode of administration. The appropriate amount can be determined by a skilled physician.

Compositions can be used alone at appropriate dosages.
10 Alternatively, co-administration or sequential administration of other agents can be desirable.

The compositions can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compositions can be administered in such oral dosage forms
15 as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they can also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or
20 intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compositions can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or four times daily. Furthermore, compositions can be
25 administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent
30 throughout the dosage regimen.

The dosage regimen utilizing the compositions is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal, hepatic and cardiovascular

function of the patient; and the particular composition thereof employed. A physician of ordinary skill can readily determine and prescribe the effective amount of the composition required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving

5 concentrations of composition within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the composition's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a composition.

The present invention also includes methods of expressing

10 HG07 in recombinant systems and then utilizing the recombinantly expressed HG07 for counter-screening. When screening compounds in order to identify potential pharmaceuticals that specifically interact with a target receptor, it is necessary to ensure that the compounds identified are as specific as possible for the target receptor. To do this, it is

15 necessary to screen the compounds against as wide an array as possible of receptors that are similar to the target receptor. Thus, in order to find compounds that are potential pharmaceuticals that interact with receptor A, it is necessary not only to ensure that the compounds interact with receptor A (the "plus target") and produce the desired pharmacological

20 effect through receptor A, it is also necessary to determine that the compounds do not interact with receptors B, C, D, *etc.* (the "minus targets"). In general, as part of a screening program, it is important to have as many minus targets as possible (see Hodgson, 1992, Bio/Technology 10:973-980, at 980). Therefore, HG07 proteins and DNA

25 encoding HG07 proteins have utility in counter-screens. That is, they can be used as "minus targets" in counter-screens in connection with screening projects designed to identify compounds that specifically interact with other G-protein coupled receptors.

The DNA of the present invention, or hybridization probes

30 based upon the DNA, can be used in chromosomal mapping studies in order to identify the chromosomal location of the HG07 gene or of genes encoding proteins related to HG07. Such mapping studies can be carried out using well-known genetic and/or chromosomal mapping techniques such as, *e.g.*, linkage analysis with respect to known chromosomal

markers or *in situ* hybridization. See, e.g., Verma et al., 1988, Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, NY. After identifying the chromosomal location of the HG07 gene or genes encoding proteins related to HG07, this information can be

5 compared with the locations of known disease-causing genes contained in genetic map data (such as the data found in the genome issue of Science (1994, 265:1981-2144). In this way, one can correlate the chromosomal location of the HG07 gene or of genes encoding proteins related to HG07 with the locations of known disease-causing genes and thus help to limit

10 the region of DNA containing such disease-causing genes. This will simplify the process of cloning such disease-causing genes. Also, once linkage between the chromosomal location of the HG07 gene or of genes encoding proteins related to HG07 and the locations of a known disease-causing gene is established, that linkage can be used diagnostically to

15 identify restriction fragment length polymorphisms (RFLPs) in the vicinity of the HG07 gene or of genes encoding proteins related to HG07. Such RFLPs will be associated with the disease-causing gene and thus can be used to identify individuals carrying the disease-causing gene.

For such chromosomal mapping studies as described herein,

20 it may be advantageous to use, in addition to the DNA of the present invention, the reverse complement of the DNA of the present invention or RNA corresponding to the DNA of the present invention.

Nucleotide sequences that are complementary to the HG07 sequences disclosed herein can be synthesized for use in antisense

25 therapy. Such antisense molecules can be DNA, stable derivatives of DNA such as phosphorothioates or methyl phosphonates, RNA, stable derivatives of RNA such as 2'-O-alkyl RNA, or other forms of HG07 antisense molecules. HG07 antisense molecules can be introduced into cells by a variety of methods, e.g., microinjection, liposome encapsulation,

30 or by expression from vectors harboring the antisense sequence. HG07 antisense therapy is expected to be particularly useful in the treatment of conditions where it is beneficial to reduce HG07 activity.

The present invention also includes antibodies to the HG07 protein. Such antibodies may be polyclonal antibodies or monoclonal

antibodies and are useful in treating disorders of the immune system that involve the inappropriate expression or activity of the HG07 protein. The antibodies of the present invention are raised against the entire HG07 protein or against suitable antigenic fragments of the protein that are

5 coupled to suitable carriers, *e.g.*, serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art. See, *e.g.*, Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and Jameson & Wolf, 1988, CABIOS (Computer Applications in the

10 Biosciences) 4:181-186.

For the production of polyclonal antibodies, HG07 protein or an antigenic fragment, coupled to a suitable carrier, is injected on a periodic basis into an appropriate non-human host animal such as, *e.g.*, rabbits, sheep, goats, rats, mice. The animals are bled periodically and sera obtained are tested for the presence of

15 antibodies to the injected antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

For the production of monoclonal antibodies, HG07 protein or an antigenic fragment, coupled to a suitable carrier, is injected into an appropriate non-human host animal as above for the production of polyclonal antibodies. In the case

20 of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, Nature 256:495-497. For a fuller description of the production of monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

Gene therapy may be used to introduce HG07 polypeptides into the cells of target organs. Nucleotides encoding HG07 polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, and polio virus based vectors. Alternatively, nucleotides

25 encoding HG07 polypeptides can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene

30

therapy with HG07 polypeptides will be particularly useful for the treatment of diseases where it is beneficial to elevate HG07 activity.

A cDNA fragment encoding full-length HG20 can be isolated from an appropriate human cDNA library by using the polymerase chain reaction (PCR) employing suitable primer pairs. Such primer pairs can be selected based upon the cDNA sequence for HG20 shown in Figure 1 as SEQ.ID.NO.:1. Suitable primer pairs would be, *e.g.*:

CGCGGATCCGCCATCATGGCACCTTCTCATCGG

(SEQ.ID.NO.:4 and

GCGGGATCCTCAAAGGTCCCATTCCGG (SEQ.ID.NO.:5).

The above primers contain BamHI sites in their 5' ends to facilitate cloning of the amplified cDNA into suitable vectors, *e.g.*, pcDNA3.1. The above primers are meant to be illustrative. One skilled in the art would recognize that a variety of other suitable primers can be designed.

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase. For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM MgCl₂, 200 μ M for each dNTP, 50 mM KCl, 0.2 μ M for each primer, 10 ng of DNA template, 0.05 units/ μ l of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 35 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20 seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W. Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael *et al.*, eds., 1990, Academic Press.

A suitable cDNA library from which a clone encoding HG07 can be isolated would be a human cDNA library made from RNA from prostate, ovary, pancreas, peripheral blood lymphocytes, or spleen. Such libraries can be prepared by methods well-known in the art.

Alternatively, several commercially available libraries would be suitable, *e.g.*, cDNA libraries from Stratagene, Inc., La Jolla, CA, USA, such as human ovary, catalog #937217, human pancreas, catalog #937208, or human fetal spleen, catalog #937205). The primary clones of such libraries can be subdivided into pools with each pool containing approximately 20,000 clones and each pool can be amplified separately.

By this method, a cDNA fragment encoding an open reading frame of 389 amino acids (SEQ.ID.NO.:2) can be obtained. This cDNA fragment can be cloned into a suitable cloning vector or expression vector. For example, the fragment can be cloned into the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, Ca). HG07 protein can then be produced by transferring an expression vector encoding HG07 into a suitable host cell and growing the host cell under appropriate conditions. HG07 protein can then be isolated by methods well known in the art.

As an alternative to the above-described PCR method, a cDNA clone encoding HG07 can be isolated from a cDNA library using as a probe oligonucleotides specific for HG07 and methods well known in the art for screening cDNA libraries with oligonucleotide probes. Such methods are described in, *e.g.*, Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K., Vol. I, II.

Oligonucleotides that are specific for HG07 and that can be used to screen cDNA libraries can be readily designed based upon the cDNA sequence of HG07 shown in Figure 1 as SEQ.ID.NO.:1 and can be synthesized by methods well-known in the art.

Genomic clones containing the HG07 gene can be obtained from commercially available human PAC or BAC libraries, *e.g.*, from Research Genetics, Huntsville, AL. Alternatively, one may prepare genomic libraries, especially in P1 artificial chromosome vectors, from which genomic clones containing the HG07 can be isolated, using probes based upon the HG07 nucleotide sequences disclosed herein. Methods of preparing such libraries are known in the art (Ioannou *et al.*, 1994, *Nature Genet.* 6:84-89).

The following non-limiting examples are presented to better illustrate the invention.

EXAMPLE 1

Tissue distribution of HG07 RNA transcripts

The data shown in Figure 4 were obtained by the use of human multi-tissue Northern blots purchased from Clontech (Palo Alto, CA). The blots were

hybridized with ^{32}P -labelled probes prepared from a 805 bp BamHI-ApaI fragment of HG07. Hybridizations were carried out using the ExpressHyb buffer supplied by Clontech under conditions suggested by Clontech. The blots were then washed in 0.1X SSC/0.1% SDS at 60°C and exposed by autoradiography.

5

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

10

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED:

1. An isolated DNA comprising nucleotides encoding a polypeptide having the amino acid sequence SEQ.ID.NO.:2.
2. The DNA molecule of claim 1 comprising a nucleotide sequence selected from the group consisting of SEQ.ID.NO.:1 and positions 158-1,324 of SEQ.ID.NO.:1.
3. A DNA molecule that hybridizes under stringent conditions to the DNA of claim 1.
4. An expression vector comprising the DNA of claim 1.
5. A recombinant host cell comprising the DNA of claim 1.
6. An isolated HG07 protein having the amino acid sequence SEQ.ID.NO.:2.
7. The isolated HG07 protein of claim 6 that is substantially free from other proteins.
8. The HG07 protein of claim 6 containing a single amino acid substitution.
9. The HG07 protein of claim 6 containing two or more amino acid substitutions where the substitutions are conservative and do not occur in positions where HG07 and the human leukotriene B4 receptor share the same amino acid.
10. A method for determining whether a substance is a potential agonist or antagonist of HG07 comprising:
- (a) transfecting cells with an expression vector encoding HG07;

(b) allowing the transfected cells to grow for a time sufficient to allow HG07 to be expressed;

(c) exposing the cells to a labeled ligand of HG07 in the presence and in the absence of the substance;

5 (d) measuring the binding of the labeled ligand to HG07; where if the amount of binding of the labeled ligand is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of HG07;

where HG07 has an amino acid sequence of SEQ.ID.NO.:2.

10

11. A method for determining whether a substance is capable of binding to HG07 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG07 in the cells;

15

(b) exposing the test cells to the substance;

(c) measuring the amount of binding of the substance to HG07;

20

(d) comparing the amount of binding of the substance to HG07 in the test cells with the amount of binding of the substance to control cells that have not been transfected with HG07;

wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to HG07;

where HG07 has an amino acid sequence of SEQ.ID.NO.:2.

25

12. A method of identifying agonists and antagonists of HG07 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG07 in the cells;

30

(b) exposing the test cells to a substance that is suspected of being an agonist or an antagonist of HG07;

(c) measuring the amount of a functional response of the test cells that have been exposed to the substance;

(d) comparing the amount of the functional response exhibited by the test cells with the amount of the functional response exhibited by control cells;

5 where if the amount of the functional response exhibited by the test cells differs from the amount of the functional response exhibited by the control cells, the substance is an agonist or antagonist of HG07;

where the control cells are cells that have not been transfected with HG07 but have been exposed to the substance or are test cells that have not been exposed to the substance;

10 where HG07 has the amino acid sequence SEQ.ID.NO.:2.

13. An antibody that binds specifically to HG07 where HG07 has an amino acid sequence of SEQ.ID.NO.:2.

1 TCTGGGAAGG AGGCCAGGAG TGGGGCAGGT CAACTGACTG GGAGCAGGGG
51 ATCTGGGTTC CAAGAAGGAG TTGTGTTTGA GGTGGGGTCT GGGTCCTCGT
101 GGAAGTCAGG ACTCCCAGGC AGAAAAGAGG CAGGCTGCAG GGAAGTAAGG
151 AGGAGGCATG GCACCTTCTC ATCGGGCATC ACAGGTGGGG TTTTGCCCCA
201 CCCCTGAACG CCCTCTGTGG CGCCTTCCAC CCACCTGTAG GCCCAGAAGG
251 ATGTCGGTCT GCTACCGTCC CCCAGGGAAC GAGACACTGC TGAGCTGGAA
301 GACTTCGCGG GCCACAGGCA CAGCCTTCCT GCTGCTGGCG GCGCTGCTGG
351 GGCTGCCTGG CAACGGTTTC GTGGTGTGGA GCTTGGCGGG CTGGCGGCTT
401 GCACGGGGGC GACCGCTGGC GGCAACGCTT GTGTTGCACC TGGCGCTGGC
451 CGACGGCGCG GTGCTGCTGC TCACGCCGTT CTTTGTGGCC TTCCTGACCC
501 GGCAGGCTTG GCCGCTGGGC CAGGCGGGCT GCAAGGCGGT GTACTACGTG
551 TGCGCGCTCA GCATGTACGC CAGCGTGCTG CTCACCGGCC TGCTCAGCCT
601 GCAGCGCTGC TTCGCAGTCA CCCGCCCTT CCTGGCGCTT CGGCTGCGCA
651 GCCCGGCCCT GGCCCGCCGC CTGCTGCTGG CGGTCTGGCT GGCCGCCCTG
701 TTGCTCGCCG TCCCGGCCGC CGTCTACCGC CACCTGTGGA GGGACCGCGT
751 ATGCCAGCTG TGCCACCCGT CGCCGGTCCA CGCCGCCGCC CACCTGAGCC
801 TGGAGACTCT GACCGCTTTC GTGCTTCCTT TCGGGCTGAT GCTCGGCTGC
851 TACAGCGTGA CGCTGGCACG GCTGCGGGGC GCCCGCTGGG GCTCCGGGCG
901 GCACGGGGCG CGGGTGGGCC GGCTGGTGAG CGCCATCGTG CTTGCCTTCG
951 GCTTGCTCTG GGCCCCCTAC CACGCAGTCA ACCTTCTGCA GGCGGTGCGA
1001 GCGCTGGCTC CACCGGAAGG GGCCTTGGCG AAGCTGGGCG GAGCCGGCCA
1051 GGCGGCGCGA GCGGGAAGGA CGGCCTTGGC CTTCTTCAGT TCTAGCGTCA
1101 ACCCGGTGCT CTACGTCTTC ACCGCTGGAG ATCTGCTGCC CCGGGCAGGT
1151 CCCCGTTTCC TCACGCGGCT CTTCGAAGGC TCTGGGGAGG CCCGAGGGGG
1201 CGGCCGCTCT AGGGAAGGGA CCATGGAGCT CCGAACTACC CCTCAGCTGA
1251 AAGTGGTGGG GCAGGGCCGC GGCAATGGAG ACCCGGGGGG TGGGATGGAG
1301 AAGGACGGTC CGGAATGGGA CCTTTGACAG CAGACCCTAC AACCTGCTGC
1351 CCTTCCCTGT CCCTTTCCAC CCCCCACCA CCCTCCAGAG GTCAGTGTTT
1401 TGGGACATTT GGGGACCCTT CTTTACTAG AGTTTGGATC TGGCTGGGTA
1451 GGATTAGTAT ACACTTGGGG CAGGCCCAGG CTCCTCCAAA CTGAGGGATT
1501 ATGAGGGTGG TGATGGTCCC TGTTAAGGAC TATTGTGTGC TTGCAAGTTG

FIG. 1

2/6

1 MAPSHRASQV GFCPTPERPL WRLPPTCRPR RMSVCYRPPG NETLLSWKTS
51 RATGTAFLLL AALLGLPGNG FVVWSLAGWR LARGRPLAAT LVLHLALADG
101 AVLLLTTPFFV AFLTRQAWPL GQAGCKAVYY VCALSMYASV LLTGLLSLQR
151 CFAVTRPFLA LRLRSPALAR RLLLAVWLAA LLLAVPAAVY RHLWRDRVCQ
201 LCHPSPVHAA AHLLETTLTA FVLPGFLMLG CYSVTLARLR GARWGSGRHG
251 ARVGRLVSAI VLAFGLLWAP YHAVNLLQAV AALAPPEGAL AKLGGAGQAA
301 RAGTTALAFF SSSVNPVLYV FTAGDLLPRA GPRFLTRLFE GSGEARGGGR
351 SREGTMELRT TPQLKVVGQG RGNGDPGGGM EKDGPEWDL

FIG.2

09831580-092801

3/6

10 30 50
CTGGGAAGGAGGCCAGGAGTGGGGCAGGTCAACTGACTGGGAGCAGGGGATCTGGGTTCC

70 90 110
AAGAAGGAGTTGTGTTTGAGGTGGGGTCTGGGTCCTCGTGGAAGTCAGGACTCCCAGGCA

130 150 170
GAAAAGAGGCAGGCTGCAGGGAAGTAAGGAGGAGGCATGGCACCTTCTCATCGGGCATCA
MetAlaProSerHisArgAlaSer

190 210 230
CAGGTGGGGTTTTGCCCCACCCCTGAACGCCCTCTGTGGCGCCTTCCACCCACCTGTAGG
GlnValGlyPheCysProThrProGluArgProLeuTrpArgLeuProProThrCysArg

250 270 290
CCCAGAAGGATGTCGGTCTGCTACCGTCCCCAGGGAACGAGACACTGCTGAGCTGGAAG
ProArgArgMetSerValCysTyrArgProProGlyAsnGluThrLeuLeuSerTrpLys

310 330 350
ACTTCGCGGGCCACAGGCACAGCCTTCTGCTGCTGGCGGCGCTGCTGGGGCTGCCTGGC
ThrSerArgAlaThrGlyThrAlaPheLeuLeuLeuAlaAlaLeuLeuGlyLeuProGly

370 390 410
AACGGTTTCGTGGTGTGGAGCTTGGCGGGCTGGCGGCTTGACGGGGGCGACCGCTGGCG
AsnGlyPheValValTrpSerLeuAlaGlyTrpArgLeuAlaArgGlyArgProLeuAla

430 450 470
GCAACGCTTGTGTTGCACCTGGCGCTGGCCGACGGCGCGGTGCTGCTGCTCACGCCGTTC
AlaThrLeuValLeuHisLeuAlaLeuAlaAspGlyAlaValLeuLeuLeuThrProPhe

490 510 530
TTTGTGGCCTTCCTGACCCGGCAGGCTTGGCCGCTGGGCCAGGCGGGCTGCAAGGCGGTG
PheValAlaPheLeuThrArgGlnAlaTrpProLeuGlyGlnAlaGlyCysLysAlaVal

550 570 590
TACTACGTGTGCGCGCTCAGCATGTACGCCAGCGTGCTGCTCACCGGCCTGCTCAGCCTG
TyrTyrValCysAlaLeuSerMetTyrAlaSerValLeuLeuThrGlyLeuLeuSerLeu

610 630 650
CAGCGCTGCTTCGCAGTCACCCGCCCTTCTGGCGCTTCGGCTGCGCAGCCCGGCCCTG
GlnArgCysPheAlaValThrArgProPheLeuAlaLeuArgLeuArgSerProAlaLeu

670 690 710
GCCC GCCGCTGCTGCTGGCGGTCTGGCTGGCCGCCCTGTTGCTCGCCGTCCCGGCCGCC
AlaArgArgLeuLeuLeuAlaValTrpLeuAlaAlaLeuLeuLeuAlaValProAlaAla

730 750 770
GTCTACGCCACCTGTGGAGGGACCGCGTATGCCAGCTGTGCCACCCGTGCGCGGTCCAC
ValTyrArgHisLeuTrpArgAspArgValCysGlnLeuCysHisProSerProValHis

FIG.3A

4/6

790 810 830
GCCGCCGCCACCTGAGCCTGGAGACTCTGACCGCTTTCGTGCTTCCTTTCGGGCTGATG
AlaAlaAlaHisLeuSerLeuGluThrLeuThrAlaPheValLeuProPheGlyLeuMet

850 870 890
CTCGGCTGCTACAGCGTGACGCTGGCACGGCTGCGGGGCGCCCGCTGGGGCTCCGGGCGG
LeuGlyCysTyrSerValThrLeuAlaArgLeuArgGlyAlaArgTrpGlySerGlyArg

910 930 950
CACGGGGCGCGGTGGGCCGGCTGGTGAGCGCCATCGTGCTTGCTTCGGCTTGCTCTGG
HisGlyAlaArgValGlyArgLeuValSerAlaIleValLeuAlaPheGlyLeuLeuTrp

970 990 1010
GCCCCCTACCACGCAGTCAACCTTCTGCAGGCGGTGCGAGCGCTGGCTCCACCGGAAGGG
AlaProTyrHisAlaValAsnLeuLeuGlnAlaValAlaAlaLeuAlaProProGluGly

1030 1050 1070
GCCTTGGCGAAGCTGGGCGGAGCCGGCCAGGCGGCGAGCGGGAACCTACGGCCTTGGCC
AlaLeuAlaLysLeuGlyGlyAlaGlyGlnAlaAlaArgAlaGlyThrThrAlaLeuAla

1090 1110 1130
TTCTTCAGTTCTAGCGTCAACCCGGTGCTCTACGTCTTCACCGCTGGAGATCTGCTGCCC
PhePheSerSerSerValAsnProValLeuTyrValPheThrAlaGlyAspLeuLeuPro

1150 1170 1190
CGGGCAGGTCCCCGTTTCCTCACGCGGCTCTTCAAGGCTCTGGGGAGGCCCGAGGGGGC
ArgAlaGlyProArgPheLeuThrArgLeuPheGluGlySerGlyGluAlaArgGlyGly

1210 1230 1250
GGCCGCTCTAGGGAAGGGACCATGGAGCTCCGAACCTACCCCTCAGCTGAAAGTGGTGGGG
GlyArgSerArgGluGlyThrMetGluLeuArgThrThrProGlnLeuLysValValGly

1270 1290 1310
CAGGGCCGCGGCAATGGAGACCCGGGGGGTGGGATGGAGAAGGACGGTCCGGAATGGGAC
GlnGlyArgGlyAsnGlyAspProGlyGlyGlyMetGluLysAspGlyProGluTrpAsp

1330 1350 1370
CTTTGACAGCAGACCCTACAACCTGCTGCCCTTCCCTGTCCCTTTCACCCCCACCCAC
Leu

1390 1410 1430
CCTCCAGAGGTCAGTGTTCTGGGACATTTGGGGACCCTTCTTTGACTAGAGTTTGGATCT

1450 1470 1490
GGCTGGGTAGGATTAGTATACACTTGGGGCAGGCCAGGCTCCTCCAAACTGAGGGATTA

1510 1530 1550
TGAGGGTGGTGATGGTCCCTGTAAAGGACTATTGTGTGCTTGCAAGTTG

FIG.3B

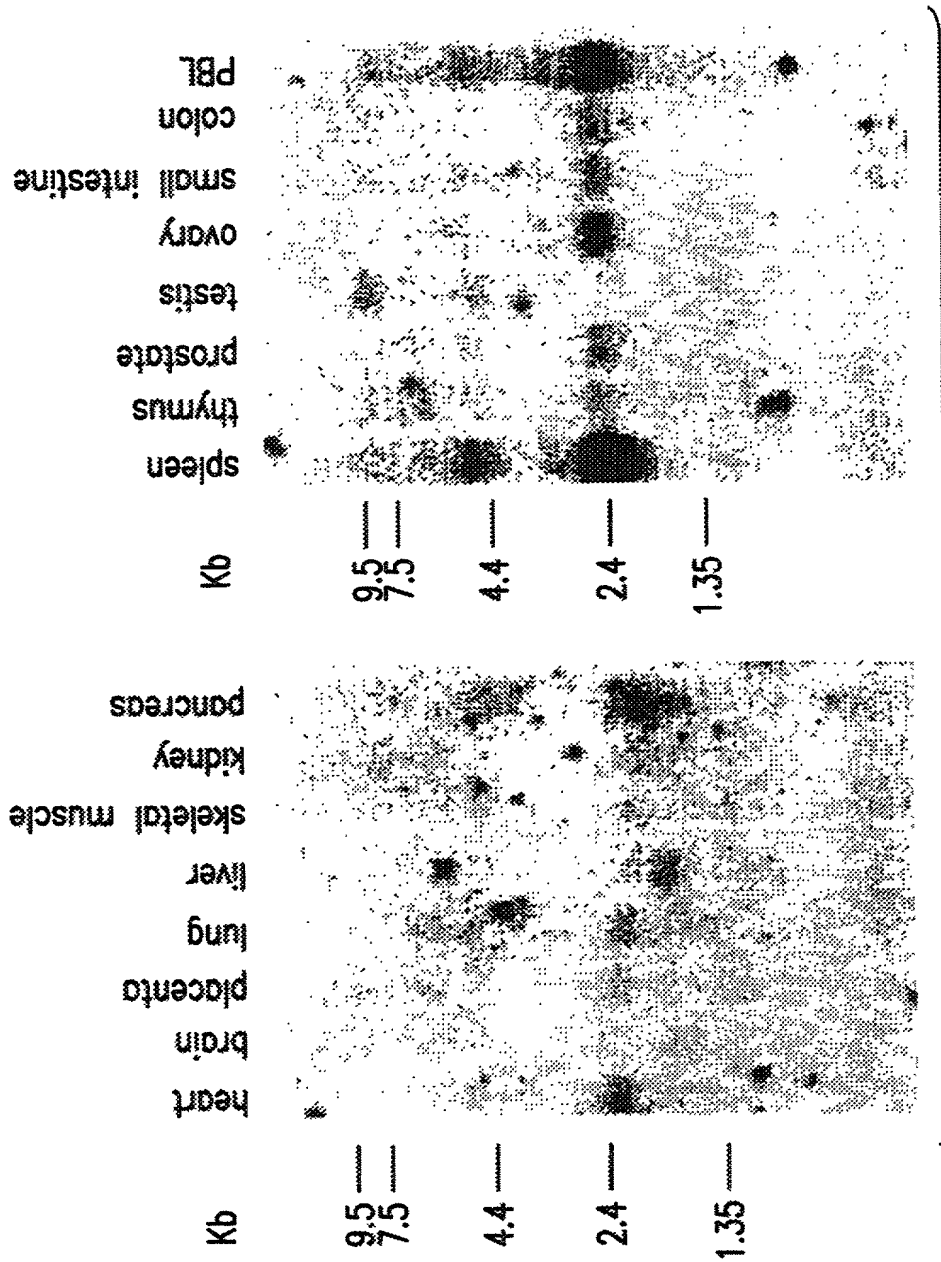


FIG.4

6/6

09/831580

10 20 30 40 49
 LTB4R MNTTSSAAPPSLGVEFISLLAIILLSVALAVGLPGNSFVWWSILK-RMQK
 |::||::| ||||:||||: |: :
 HG07 TCRPRRMSVCYRPPGNETLLSWKTSRATGTAFLLLAALL--GLPGNGFVWWSLAGWRLAR
 30 40 50 60 70 80
 50 60 70 80 90 100
 LTB4R -RSVTALMVLNLALADLAVLLTAPFFLHFLAOGTWSFGLAGCRLCHYVCGVSMYASVLLI
 |::|:|:||||| ||| :||: ||:: :| :| |||: :|||:|||||||
 HG07 GRPLAATLVHLALADGAVLLLTPFFVAFLTRQAWPLGQAGCKAVYYVCALSMYASVLLT
 90 100 110 120 130 140
 110 120 130 140 150 160
 LTB4R TAMS LDRSLAVARPFVSQKLR TKAMARRVLAGIWVLSFLLATPVLAYRTVVPWKTNM-SL
 :||:|:|:||||: :||: |::||:|: :|: :||:|: :||: |: : :|
 HG07 GLLSLQRCFAVTRPFLALRLRSPALARRLLLAVWLAALLAVPAAYRHL--WRDRVQQL
 150 160 170 180 190 200
 170 180 190 200 210 220
 LTB4R CFPRYPSEGHRAFLIFEAVTGFLLPFLAVVASYS DIGRR LQARRF---RRSRRTGRLVV
 | :|| | | | :|:|:|:| || ::: || ||:: |: |: |:|:|
 HG07 C---HPSPVHAAHLSLETLTAFVLPFGLMLGCYSVTLARLRGARWGSGRHGARVGRVLS
 210 220 230 240 250
 230 240 250 260 270 280
 LTB4R LIILTFAAFWLPYHVVNLA EAGRALAGQAAGLGLVGKRLSLARNVLIVLAF LSSSVNPVL
 |:|:|: :| |||:| || | || :|:|:| : || :||:|||||||
 HG07 AIVLAFGLLWAPYHAVNLLQAVAALAPPEGALAKLGAGQAARAGTTALAFFSSSVNPVL
 260 270 280 290 300 310
 290 300 310 320 330 340
 LTB4R YACAGGGLVRSAGVGFVAKLLEGTGSEASSTRGGSLGQTARSGPAALEPGPSESLTASS
 |: :| |: || |:|:|:| || ||| |:| |:| |:| :|
 HG07 YVFTAGDLLPRAGPRFLTRLFEGSG-EA----RGG--GRS-REGTMELRTTPQLKVVGQG
 320 330 340 350 360 370
 350
 LTB4R PLKLNELN
 HG07 RGNGDPGGGMEKDGPEWDL
 380 390

FIG.5

09831580-092801

**DECLARATION AND
POWER OF ATTORNEY
FOR UTILITY OR DESIGN
PATENT APPLICATION
(37 CFR 1.63)**☐ Declaration
Submitted
with Initial
Filing

OR

☒ Declaration
Submitted after Initial
Filing (surcharge
(37 CFR 1.16 (e))
required)

Attorney Docket Number 20332P

First Named Inventor Liu, et al.

COMPLETE IF KNOWN

Application Number 09/831,580

Filing Date May 11, 2001

Group Art Unit

Examiner Name

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

G PROTEIN-COUPLED RECEPTOR RESEMBLING THE LEUKOTRIENE B4 RECEPTOR

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 05/11/2001 as United States Application Number or PCT International

Application Number 09/831,580 and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Attorney Docket Number	Priority Claimed?	
				YES	NO
PCT/US99/26303	PCT	11/08/1999	20332-PCT	<input checked="" type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	Attorney Docket Number
60/108,111	11/12/1998	20332PV

DECLARATION AND POWER OF ATTORNEY for Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information known to me to be material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Application Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
60/108,111	11/12/1998	
PCT/US99/26303	11/08/1999	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint, respectively and individually, as my attorneys or agents with full power of substitution and revocation, the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

☐ Customer Number

OR

☒ Registered practitioner(s) name/registration number listed below

Place Customer Number
Bar Code Label here

Name	Registration Number	Name	Registration Number
Joseph A. Coppola	38,413	Jack L. Tribble	32,633

Direct all correspondence to: ☒ Customer Number or Bar Code Label

000210

Name	Joseph A. Coppola				
Address	Merck & Co., Inc. - Patent Department				
Address	P.O. Box 2000, RY60-30				
City	Rahway	State	NJ	ZIP	07065-0907
Country	USA	Telephone	(732)594-6734	Fax	(732)594-4720

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])		Family Name or Surname	
QINGYUN		LIU	
Inventor's Signature	Date		Aug. 13, 2001
Residence: City	North Wales PA	State	PA
		Country	US
		Citizenship	CN
Post Office Address	Merck & Co., Inc., P.O. Box 2000		
City	Rahway	State	NJ
		ZIP	07065-0907

☒ Additional inventors are being named on the 1 supplemental Additional Inventors(s) sheet(s) PTO/SB/02A attached hereto.

DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S)
Supplemental Sheet

SEP 28 2001

Name of Additional Joint Inventor, if any:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])

Family Name or Surname

RUIPING

WANG

Inventor's
Signature

Date

Aug 13, 2001

Residence:
City

Maple Glen

PA

State PA

Country US

Citizenship CN

Post Office
Address

Merck & Co., Inc., P.O. Box 2000

City

Rahway

State NJ

ZIP

07065-0907

Name of Additional Joint Inventor, if any:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])

Family Name or Surname

WENDY J.

BAILEY

Inventor's
Signature

Date

Aug 13, 2001

Residence:
City

Fort Washington

PA

State PA

Country US

Citizenship US

Post Office
Address

MICHAEL

City

Rahway

State NJ

ZIP

07065-0907

Name of Additional Joint Inventor, if any:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])

Family Name or Surname

MICHAEL

DAVIDOFF

Inventor's
Signature

Date

Residence:
City

Minneapolis

State MN

Country US

Citizenship US

Post Office
Address

Merck & Co., Inc., P.O. Box 2000

City

Rahway

State NJ

ZIP

07065-0907

Name of Additional Joint Inventor, if any:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])

Family Name or Surname

Inventor's
Signature

Date

Residence:
City

State

Country

Citizenship

Post Office
Address

Merck & Co., Inc., P.O. Box 2000

City

Rahway

State NJ

ZIP

07065-0907

**DECLARATION AND
POWER OF ATTORNEY
FOR UTILITY OR DESIGN
PATENT APPLICATION
(37 CFR 1.63)**☐ Declaration
Submitted
with Initial
Filing

OR

☒ Declaration
Submitted after Initial
Filing (surcharge
(37 CFR 1.16 (e))
required)

Attorney Docket Number 20332P

First Named Inventor Liu, et al.

COMPLETE IF KNOWN

Application Number 09/831,580

Filing Date May 11, 2001

Group Art Unit

Examiner Name

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

G PROTEIN-COUPLED RECEPTOR RESEMBLING THE LEUKOTRIENE B4 RECEPTOR

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 05/11/2001 as United States Application Number or PCT International

Application Number 09/831,580 and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Attorney Docket Number	Priority Claimed?	
				YES	NO
PCT/US99/26303	PCT	11/08/1999	20332-PCT	<input checked="" type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	Attorney Docket Number
60/108,111	11/12/1998	20332PV

DECLARATION AND POWER OF ATTORNEY for Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information known to me to be material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Application Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
60/108,111	11/12/1998	
PCT/US99/26303	11/08/1999	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint, respectively and individually, as my attorneys or agents with full power of substitution and revocation, the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

☐ Customer Number

OR

☒ Registered practitioner(s) name/registration number listed below

Place Customer Number
Bar Code Label here

Name	Registration Number	Name	Registration Number
Joseph A. Coppola	38,413	Jack L. Tribble	32,633

Direct all correspondence to: ☒ Customer Number or Bar Code Label

000210

Name	Joseph A. Coppola				
Address	Merck & Co., Inc. - Patent Department				
Address	P.O. Box 2000, RY60-30				
City	Rahway	State	NJ	ZIP	07065-0907
Country	USA	Telephone	(732)594-6734	Fax	(732)594-4720

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:

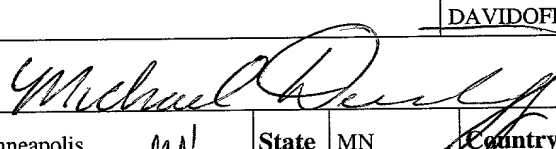
☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])		Family Name or Surname			
QINGYUN		LIU			
Inventor's Signature				Date	
Residence: City	North Wales	State	PA	Country	US
				Citizenship	CN
Post Office Address	Merck & Co., Inc., P.O. Box 2000				
City	Rahway	State	NJ	ZIP	07065-0907
<input checked="" type="checkbox"/> Additional inventors are being named on the <u>1</u> supplemental Additional Inventors(s) sheet(s) PTO/SB/02A attached hereto.					

DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S)
Supplemental Sheet

SEP 28 2001

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
RUIPING				WANG			
Inventor's Signature				Date			
Residence: City		Maple Glen		State PA		Country US	
Post Office Address		Merck & Co., Inc., P.O. Box 2000					
City		Rahway		State NJ		ZIP 07065-0907	
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
WENDY J.				BAILEY			
Inventor's Signature				Date			
Residence: City		Fort Washington		State PA		Country US	
Post Office Address		MICHAEL					
City		Rahway		State NJ		ZIP 07065-0907	
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
MICHAEL				DAVIDOFF			
Inventor's Signature				Date		8/29/2001	
Residence: City		Minneapolis MN		State MN		Country US	
Post Office Address		Merck & Co., Inc., P.O. Box 2000					
City		Rahway		State NJ		ZIP 07065-0907	
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
Inventor's Signature				Date			
Residence: City				State		Country	
Post Office Address		Merck & Co., Inc., P.O. Box 2000					
City		Rahway		State NJ		ZIP 07065-0907	